named sequences, SEQ ID NOS:1-417, in computer readable form, and a paper copy of the sequence information which has been printed from the electronic copy from the computer disk. Typographical errors have also been corrected.

The information contained in the referenced electronic copy was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Justification for the amendment to page 199, line 34, which omits the terminal "Glu" residue from SEQ ID NO:166, corrects a typographical error. The nucleotide sequence (SEQ ID NO:165), on line 33 directly above the corresponding amino acid sequence, contains a two nucleotide gap at the position above the omitted "Glu". The nucleotide sequence at this position is "...G__gt..., where GGT codes for Gly, not the "Glu" depicted. This gap in the nucleotide sequence is filled in subsequently in SEQ ID NO:167 on line 37. That this is indeed a typographical error can be seen by comparison with the next example, on page 200, lines 9-15, where the amino acid residue corresponding to the two nucleotide gap is absent.

The Examiner's attention is drawn to those sequences in FIG. 40 which have been modified for the Sequence Listing (SEQ ID NOS:269, 276, 280, 282, 283, 286, 289, 291, 295, 299, 301 and 303). The numbers above the top line of germline sequence ("GL") refer to codon numbers, which correspond to those codons found in the "VH251.GL" germline sequence found in its entirety in FIG. 66A.1 and continued in FIG. 66A.2. SEQ ID NO:269 represents the contiguous sequence of the "GL" codons labeled 28-32. Similarly, SEQ ID NOS:276, 280, 282, 283, 286, 289, 291, 295, 299, 301 and 303 correspond to only the contiguous segments of the variable region cDNAs from codons 28-32 each time a nucleotide substitution occurred in this region.

Following the same logic, the sequences of the J2-J6 regions (SEQ ID NOS:270, 272, 275, 294, 298 and 304) have been truncated to exclude the non-contiguous codon 108 at the 3' end. Likewise, the CDR III sequences from the variable region cDNAs (SEQ ID NOS:271, 273, 274, 277-279, 281, 284, 285, 287, 288, 290, 292, 293, 296, 297, 300, 302 and 305) begin at the 5' end of contiguous nucleotides and extend through the

respective portion of the appropriate J chain, with any noted nucleotide substitutions, to codon number 102 of the J chain, and exclude the 'terminal" codon 108.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Andrew T. Serafini
Reg. No. 41,303

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
ATS:adm
PA 3175539 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at page 1, line 6 has been amended as follows:

This application is a continuation of USSN 08/758,417, filed November 12, 1998, and is a continuation-in-part of U.S. Serial Number 08/728,463 filed October 10, 1996, which is a continuation-in-part of U.S. Serial No. 08/544,404 filed 10 October 1995, which is a continuation-in-part of U.S. Serial No. 08/352,322 filed 7 December 1994, which is a continuation-in-part of U.S. Serial No. 08/209,741 filed March 9, 1994, which is a continuation–in-part of U.S. Serial No. 08/165,699 filed December 10, 1993, which is a continuation–in-part of [os] U.S. Serial No. 08/161,739 filed December 3, 1993, which is a continuation-in-part of U.S. Serial No. 08/155,301 filed November 18, 1993, which is a continuation-in-part of U.S. Serial No. 08/096,762 filed July 22, 1993, which is a continuation-in-part of U.S. Serial No. 08/053,131 filed April 26, 1993, which is a continuation-in-part of U.S. Serial No. 07/990,860 filed December 16, 1992, which is a continuation-in-part of U.S. Serial No. 07/904,068 filed June 23, 1992, which is a continuation-in-part of U.S. Serial No. 07/853,408 filed March 18, 1992, which is a continuation-in-part of U.S. Serial No. 07/810,279 filed December 17, 1991, which is a continuation-in-part of U.S. Serial No. 07/575,962 filed August 31, 1990 (now abandoned), which is a continuation-in-part of U.S. Serial No. 07/574,748 filed August 29, 1990 (now abandoned). This application claims foreign priority benefits under Title 35, United States Code, Section 119, to PCT Application No. PCT/US91/06185 (which corresponds to U.S. Serial No. 07/834,539 filed February 5, 1992) and PCT Application No. PCT/US92/10983.

The paragraph beginning at page 15, line 29 has been amended as follows:

Fig. 40 shows aligned variable region sequences of 23 randomly-chosen cDNAs (SEQ ID NOS:269, 271, 273, 274, 276-293, 296, 297 and 299-305) generated from mRNA obtained from lymphoid tissue of HC1 transgenic mice immunized with human carcinoembryonic antigen (CEA) as compared to the germline transgene sequence (top line)(SEQ ID NO:269); on each line nucleotide changes relative to germline sequence are shown. The regions corresponding to heavy chain CDR1, CDR2, and CDR3 are indicated. Non-germline encoded nucleotides are shown in capital letters. J chain sequences for J2, J3, J4, J5, and J6 are given as SEQ ID NOS:270, 272, 275, 294 and 298, respectively.

The paragraph beginning at page 16, line 1 has been amended as follows:

Fig. 41 shows the nucleotide sequence (SEQ ID NO:306) of a human DNA fragment, designated vk65.3, containing a V_κ gene segment; the deduced amino acid sequences (SEQ ID NO:307) of the V_κ coding regions are also shown; slicing and recombination signal sequences (heptamer/nonamer) are shown boxed.

The paragraph beginning at page 16, line 6 has been amended as follows:

Fig. 42 shows the nucleotide sequence (SEQ ID NO:308) of a human DNA fragment, designated vk65.5, containing a V_κ gene segment; the deduced amino acid sequences (SEQ ID NO:309) of the V_κ coding regions are also shown; splicing and recombination signal sequences (heptamer/nonamer) are shown boxed.

The paragraph beginning at page 16, line 11 has been amended as follows:

Fig. 43 shows the nucleotide sequence (SEQ ID NO:310) of a human DNA fragment, designated vk65.8, containing a V_κ gene segment; the deduced amino acid sequences (SEQ ID NO:311) of the V_κ coding regions are also shown; splicing and recombination signal sequences (heptamer/nonamer) are shown boxed.

The paragraph beginning at page 16, line 16 has been amended as follows:

Fig. 44 shows the nucleotide sequence (SEQ ID NO:312) of a human DNA fragment, designated vk65.15, containing a V_κ gene segment; the deduced amino acid sequences (SEQ ID NO:313) of the V_κ coding regions are also shown; splicing and recombination signal sequences (heptamer/nonamer) are shown boxed.

The paragraph beginning at page 16, line 27 has been amended as follows:

Fig. 47 shows the DNA sequences (SEQ ID NOS:314-322) of 10 cDNAs amplified by PCR to amplify transcripts having a human VDJ and a murine constant region sequence.

The paragraph beginning at page 18, line 4 has been amended as follows:

Fig. 61 shows a nucleotide sequence (SEQ ID NO:313) of mouse heavy chain locus α constant region gene.

The paragraph beginning at page 19, line 8 has been amended as follows:

Fig. 65 shows chimeric human/mouse immunoglobulin heavy chains generated by trans-switching. cDNA clones of trans-switch products were generated by reverse transcription and PCR amplification of a mixture of spleen and lymph node RNA isolated from a hyperimmunized HC1 transgenic-JHD mouse (#2357, see legend to Fig. 63 for description of animal and immunization schedule). The partial nucleotide sequence of 10 randomly picked clones is shown (SEQ ID NOS:324-332). Lower case letters indicate germline encoded, capital letters indicate nucleotides that cannot be assigned to known germline sequences; these may be somatic mutations, N nucleotides, or truncated D segments. Both face type indicates mouse γ sequences.

The paragraph beginning at page 19, line 20 has been amended as follows:

Figs. 66A and 66B show that the rearranged VH251 transgene undergoes somatic mutation in a hyperimmunized mouse. The partial nucleotide sequence of IgG heavy chain variable region cDNA clones from CH1 line 26 mice exhibiting Fig. 66A primary and Fig. 66B secondary responses to antigen. Germline sequence is shown at the top(SEQ ID NO:333); nucleotide changes from germline are given for each clone. A period indicates identity with germline sequence, capital letters indicate no identified germline origin. The sequences are grouped according to J segment usage. The germline sequence of each of the J segments [if] is shown (FIG. 66A.2, SEQ ID NOS:334, 338, 340 and 347; FIG. 66B2, SEQ ID NOS:350, 352, 360 and 362). Lower case letters within CDR3 sequences indicate identity to known D segment included in the HC1 transgene. The assigned D segments are indicated at the end of each sequence. Unassigned sequences could be derived from N region addition or somatic mutation; or in some cases they are simply too short to distinguish random N nucleotides from known D segments. Fig. 66A primary response: 13 randomly picked VH251- γ1 cDNA clones (SEQ ID NOS:335-337, 339, 341-346, 348 and 349). A 4 week old female HC1 line 26-JHD mouse (#2599) was given a single injection of KLH and complete Freunds adjuvant; spleen cell RNA was isolated 5 days later. The overall frequency of somatic mutations within the V segment is 0.06% (2/3,198 bp). Fig. 66B secondary response: 13 randomly picked VH251- γ1 cDNA clones (SEQ ID NOS:351, 353, 355-359, 361, and 363). A 2 month old female HC1 line 26-JHD mouse (#3204) was given 3 injections of HEL and Freunds adjuvant over one month (a primary injection with complete

adjuvant and boosts with incomplete at one week and 3 weeks); spleen and lymph node RNA was isolated 4 months later. The overall frequency of somatic mutations within the V segment is 1.6% (52/3,198 bp).

The paragraph beginning at page 20, line 11 has been amended as follows:

Figs. 67A and 67B show that extensive somatic mutation is confined to γ1 sequences: somatic mutation and class switching occur within the same population of B cells. Partial nucleotide sequence of VH251 cDNA clones isolated from spleen and lymph node cells of HC1 line 57 transgenic-JHD mouse (#2357) hyperimmunized against CEA (see Fig. 63 for immunization schedule). Fig. 67A: IgM: 23 randomly picked VH251-μ cDNA clones (SEQ ID NO:364). Nucleotide sequence of 156 bp segment including CDRs 1 and 2 surrounding residues(SEQ ID NOS:364-368). The overall level of somatic mutation is 0.1% (5/3,744 bp). Fig. 67B: IgG: 23 randomly picked VH251- γ 1 cDNA clones(SEQ ID NO:333). Nucleotide sequence of segment including CDRs 1 through 3 and surrounding residues (SEQ ID NOS:369-391). J chain sequences for J2, J3, J4, J5, and J6 are given as SEQ ID NOS:350, 352, 354, 360, and 362, respectively. The overall frequency of somatic mutation within the V segment is 1.1% (65/5,658 bp). For comparison with the μ sequences in Fig. 67A: the mutation frequency for the first 156 nucleotides is 1.1% (41/3,588 bp). See legend to Figs. 66A and 66B for explanation of symbols.

The paragraph beginning at page 20, line 28 has been amended as follows:

Fig. 68 indicates that [VH51P1 and VH56P1] VH56P1 (SEQ ID NO:392, VH51P1 (SEQ ID NO:410) and VH4.21 (SEQ ID NO:412) show extensive somatic mutation [of] in an unimmunized mouse. The partial nucleotide sequence of IgG heavy chain variable region cDNA clones (SEQ ID NOS:393-409, 411 and 413) from a 9 week old, unimmunized female HC2 line 2250 transgenic-JHD mouse (#5250). The overall frequency of somatic mutation with the 19 VH56p1 segments is 2.2% (101/4,674 bp). The overall frequency of somatic mutation within the single VH51p1 segment is 2.0% (5/246 bp). See legend to Figs. 66A and 66B for explanation of symbols.

The paragraph beginning at page 22, line 30 has been amended as follows:

Fig. 77A depicts the nucleotide sequence (SEQ ID NO:416) [and restriction map] of pGP2b plasmid vector.

The paragraph beginning at page 24, line 12 has been amended as follows:

Table 1 depicts the sequence of vector pGPe (SEQ ID NO:72).

The paragraph beginning at page 24, line 13 has been amended as follows:

Table 2 depicts the sequence of gene V_H49.8 (SEQ ID NOS:79-80).

The paragraph beginning at page 24, line 23 has been amended as follows:

Table 7 depicts the length of CDR3 peptides from transcripts with in–frame VDJ joints (SEQ ID NO:116-145) in the pHC1 transgenic mouse and in human PBL.

The paragraph beginning at page 24, line 33 has been amended as follows:

Table 11 [shows human variable region usage in hybridomas from transgenic mice] shows transgenic V and J segment usage.

The paragraph beginning at page 24, line 35 has been amended as follows:

Table 12 shows [transgene V and J segment usage] the occurrence of somatic mutation in the HC2 heavy chain transgene in transgenic mice.

The paragraph beginning at page 24, line 36 has been amended as follows:

Table 13 shows [the occurrence of somatic mutation in the HC2 heavy chain transgene in transgenic mice] human variable region usage in hybridomas from transgenic mice.

The paragraph beginning at page 36, line 35 has been amended as follows:

A recognizable D region sequence is generally at least about eight consecutive nucleotides corresponding to the sequence present in a D region gene segment of a heavy chain transgene and/or the amino acid sequence encoded by such D region nucleotide sequence. For example, if a transgene includes the D region gene DHQ52, a transgene-encoded mRNA containing the sequence 5'-TAACTGGG-3' located in the V region between a V gene segment sequence and a J gene segment sequence is recognizable as containing a D region sequence, specifically a DHQ52 sequence. Similarly, for example, if a transgene includes the D region gene DHQ52, a transgene-encoded heavy chain polypeptide containing

the amino acid sequence -DAF- located in the V region between a V gene segment amino acid sequence and a J gene segment amino acid sequence may be recognizable as containing a D region sequence, specifically a DHQ52 sequence. However, since D region segments may be incorporated in VDJ joining to various extents and in various reading frames, a comparison of the D region area of a heavy chain variable region to the D region segments present in the transgene is necessary to determine the incorporation of particular D segments. Moreover, potential exonuclease digestion during recombination may lead to imprecise V-D and D-J joints during V-D-J recombination.

The paragraph beginning at page 37, line 21 has been amended as follows:

However, because of somatic mutation and N-region addition, some D region sequences may be recognizable but may not correspond identically to a consecutive D region sequence in the transgene. For example, a nucleotide sequence 5'-CTAAXTGGGG-3' (SEQ ID NO:1), where X is A, T, or G, and which is located in a heavy chain V region and flanked by a V region gene sequence and a J region gene sequence, can be recognized as corresponding to the DHQ52 sequence 5'-CTAACTGGG-3'. Similarly, for example, the polypeptide sequences -DAFDI-(SEQ ID NO:2), -DYFDY-(SEQ ID NO:3), or -GAFDI-(SEQ ID NO:4) located in a V region and flanked on the amino-terminal side by an amino acid sequence encoded by a transgene V gene sequence and flanked on the carboxyterminal side by an amino acid sequence encoded by a transgene J gene sequence is recognizable as a D region sequence.

The paragraph beginning at page 38, line 28 has been amended as follows:

The term "substantial similarity" denotes a characteristic of [an] a polypeptide sequence , wherein the polypeptide sequence has at least 80 percent similarity to the reference sequence. The percentage of sequence similarity is calculated by scoring identical amino acids or positional conservative amino acid substitutions as similar. A positional conservative amino acid substitution is one that can result from a single nucleotide substitutiion; a first amino acid replaced by a second amino acid where a codon for the first amino acid and a codon for the second amino acid can differ by a single nucleotide substitution. thus, for example, the sequence -Lys-Glu-Arg-Val- (SEQ ID NO:5) is substantially similar to the sequence -Asn-Asp-Ser-Val-(SEQ ID NO:6), since the codon sequence -AAA-GAA-AGA-GUU-(SEQ ID NO:7) can be mutated to -AAC-GAC-AGC-GUU-(SEQ ID NO:8) by introducing only 3 substitution mutations, single nucleotide

substitutions in three of the four original codons. The reference sequence may be a subset of a larger sequence, such as an entire D gene; however, the reference sequence is at least 4 amino residues long. Typically, the reference sequence is at least 5 amino acids, and preferably the reference sequence is 6 amino acids or more.

The paragraph beginning at page 48, line 30 has been amended as follows:

The switch (S) region of the μ gene, S_μ, is located about 1 to 2 kb 5' to the coding sequence and is composed of numerous tandem repeats of sequences of the form $(GAGCT)_n(GGGGT)$ (SEQ ID NOS:9-24), where n is usually 2 to 5 but can range as high as 17. (See T. Nikaido et al. Nature 292:845-848 (1981))

The paragraph beginning at page 102, line 9 has been amended as follows:

The NotI digested DNA is then fractionated by pulsed field gel electrophoresis as described by Anand et al., Nucl. Acids Res. 17:3425-3433 (1989). Fractions enriched for the NotI fragment are assayed by Southern hybridization to detect one or more of the sequences encoded by this fragment. Such sequences include the heavy chain D segments, J segments, μ and γ1 constant regions together with representatives of all 6 VH families (although this fragment is identified as a 670 kb fragment from HeLa cells by Berman et al. (1988), supra., we have found it to be as 830 kb fragment from human placental [an] and sperm DNA). Those fractions containing this NotI fragment (see Fig. 4) are pooled and cloned into the NotI site of the vector pYACNN in [Yeast] yeast cells. Plasmid pYACNN is prepared by digestion of pYAC-4 Neo (Cook et al., Nucleic Acids Res. 16: 11817 (1988)) with EcoRI and ligation in the presence of the oligonucleotide 5' - AAT TGC GGC CGC - 3'(SEQ ID NO:25).

The paragraph beginning at page 103, line 34 has been amended as follows:

The oligonucleotides are:

oligo-1 5'- CTT GAG CCC GCC TAA TGA GCG GGC TTT TTT TTG CAT ACT GCG GCC - 3'(SEQ ID NO:26)

oligo-2 5'- GCA ATG GCC TGG ATC CAT GGC GCG CTA GCA TCG ATA TCT AGA GCT CGA GCA - 3'(SEQ ID NO:27)

oligo-3 5'- TGC AGA TCT GAA TTC CCG GGT ACC AAG CTT ACG CGT ACT

AGT GCG GCC GCT - 3'(SEQ ID NO:28)

oligo-4 5'- AAT TAG CGG CCG CAC TAG TAC GCG TAA GCT TGG TAC CCG GGA ATT - 3'(SEQ ID NO:29)

oligo-5 5'- CAG ATC TGC ATG CTC GAG CTC TAG ATA TCG ATG CTA GCG CGC CAT GGA TCC - 3'(SEQ ID NO:30)

oligo-6 5'- AGG GCA TTG CGG CCG CAG TAT GCA AAA AAA AGC CCG CTC ATT AGG CGG GCT - 3'(SEQ ID NO:31)

The paragraph beginning at page 104, line 28 has been amended as follows:

The following adapter oligonucleotides are ligated to the thus digested pGP1 to form pGP2.

5' CGC GTG GCC GCA ATG GCC A 3'(SEQ ID NO:32)
5' CTA GTG GCC ATT GCG GCC A 3'(SEQ ID NO:33)

The paragraph beginning at page 105, line 1 has been amended as follows:

The heavy chain region 3' enhancer described by Petterson et al., Nature 344:165-168 (1990), which is incorporated herein by reference) is isolated and cloned. The rat IGH 3' enhancer sequence is PCR amplified by using the following oligonucleotides:

5' CAG GAT CCA GAT ATC AGT ACC TGA AAC AGG GCT TGC 3'(SEQ ID NO:34)
5' GAG CAT GCA CAG GAC CTG GAG CAC ACA CAG CCT TCC 3'(SEQ ID NO:35)

The paragraph beginning at page 105, line 18 has been amended as follows:

A 6.3 kb BamHI/HindIII fragment that includes all human J segments (Matsuda et al., EMBO J., 7:1047-1051 (1988); Ravetech et al.[m] Cell, 27:583-591 (1981), which are incorporated herein by reference) is isolated from a human genomic DNA library using the oligonucleotides GGA CTG TGT CCC TGT GTG ATG CTT TTG ATG TCT GGG GCC AAG (SEQ ID NO:36).

The paragraph beginning at page 105, line 24 has been amended as follows:

An adjacent 10 kb HindIII/BamII fragment that contains enhancer, switch and constant region coding exons (Yasui et al., Eur. J. Immunol. 19:1399-1403 (1989)) is

similarily isolated using the oligonucleotide : CAC CAA GTT GAC CTG CCT GGT CAC AGA CCT GAC CAC CTA TGA (SEQ ID NO:37).

The paragraph beginning at page 105, line 29 has been amended as follows:

An adjacent 3' 1.5 kb BamHI fragment is similarly isolated using clone pMUM insert as a probe (pMUM is 4 kb EcoRI/HindIII fragment isolated from a human genomic DNA library with oligonucleotide:

CCT GTG GAC CAC CGC CTC CAC CTT CAT CGT CCT CTT CCT CCT (SEQ ID NO:38) mu membrane exon 1) and cloned into pUC19.

The paragraph beginning at page 107, line 10 has been amended as follows:

Phage clones containing the γ-1 region are identified and isolated using the following oligonucleotide which is specific for the third exon of γ-I (CH3).

5' TGA GCC ACG AAG ACC CTG AGG
TCA AGT TCA ACT GGT ACG TGG 3' (SEQ ID NO:39)

The paragraph beginning at page 108, line 5 has been amended as follows:

The strategy for cloning the human D segments is depicted in Fig. 13. Phage clones from the human genomic library containing D segments are identified and isolated using probes specific for diversity region sequences (Ichihara et al., EMBO J. 7:4141-4150 (1988)). The following oligonucleotides are used:

DXP1: 5'- TGG TAT TAC TAT GGT TCG GGG AGT TAT TAT AAC CAC AGT GTC - 3'(SEQ ID NO:40)

DXP4: 5'- GCC TGA AAT GGA GCC TCA GGG CAC AGT GGG CAC GGA CAC TGT - 3'(SEQ ID NO:41)

DN4: 5'- GCA GGG AGG ACA TGT TTA GGA TCT GAG GCC GCA CCT GAC ACC - 3'(SEQ ID NO:42)

The paragraph beginning at page 109, line 9 has been amended as follows:

An unrearranged V segment corresponding to that identified as the V segment contained in the hybridoma of Newkirk et al., J. Clin. Invest. 81:1511-1518 (1988), which is incorporated herein by reference, is isolated using the following oligonucleotide:

5'- GAT CCT GGT TTA GTT AAA GAG GAT TTT ATT CAC CCC TGT GTC - 3'(SEQ ID NO:43)

The paragraph beginning at page 110, line 19 has been amended as follows:

A 6.3 kb BamHI/HindIII fragment containing human J segments (see fragment (a) in Fig. 9) is cloned into MluI/SpeI digested pHIG5' using the following adapters:

5' GAT CCA AGC AGT 3' '(SEQ ID NO:44)
5' CTA GAC TGC TTG 3"(SEQ ID NO:45)

5' CGC GTC GAA CTA 3"(SEQ ID NO:46)

5' AGC TTA GTT CGA 3"(SEQ ID NO:47)

The paragraph beginning at page 111, line 21 has been amended as follows:

The construction of pEμ1 is depicted in Fig. 16. The mouse heavy chain enhancer is isolated on the XbaI to EcoRI 678 bp fragment (Banerji et al., Cell 33: 729-740 (1983)) from phage clones using oligo:

5' GAA TGG GAG TGA GGC TCT CTC ATA CCC TAT TCA GAA CTG ACT 3'(SEQ ID NO:48)

The paragraph beginning at page 112, line 34 has been amended as follows:

Clones from each library were probed with the C_κ specific oligo:

5' GAA CTG TGG CTG CAC CAT CTG TCT TCA TCT TCC CGC CAT CTG 3'(SEQ ID NO:49)

The paragraph beginning at page 114, line 15 has been amended as follows:

Cells from human hybridoma, or lymphoma, or other cell line that synthesizes either cell surface or secreted or both forms of IgM with a κ light chain are used for the isolation of polyA+ RNA. The RNA is then used for the synthesis of oligo dT primed cDNA using the enzyme reverse transcriptase (for general methods see, Goodspeed et al. (1989) Gene 76: 1; Dunn et al. (1989) J. Biol. Chem. 264: 13057). The single stranded cDNA is then isolated and G residues are added to the 3' end using the enzyme polynucleotide terminal

transferase. The G-tailed single-stranded cDNA is then purified and used as template for second strand synthesis (catalyzed by the enzyme DNA polymerase) using the following oligonucleotide as a primer:

5' - GAG GTA CAC TGA CAT ACT GGC ATG CCC CCC CCC CCC - 3'(SEQ ID NO:50)

The paragraph beginning at page 115, line 2 has been amended as follows:

The double stranded cDNA described in part A is denatured and used as a template for a third round of DNA synthesis using the following oligonucleotide primer:

5' - GTA CGC CAT ATC AGC TGG ATG AAG TCA TCA GAT GGC GGG AAG ATG AAG ACA GAT GGT GCA - 3'(SEQ ID NO:51)

The paragraph beginning at page 115, line 10 has been amended as follows:

This primer contains sequences specific for the constant portion of the κ light chain message (TCA TCA GAT GGC GGG AAG ATG AAG ACA GAT GGT GCA) (SEQ ID NO:52) as well as unique sequences that can be used as a primer for the PCR amplification of the newly synthesized DNA strand (GTA CGC CAT ATC AGC TGG ATG AAG) (SEQ ID NO:53). The sequence is amplified by PCR using the following two oligonucleotide primers:

5' - GAG GTA CAC TGA CAT ACT GGC ATG - 3'(SEQ ID NO:54)
5' - GTA CGC CAT ATC AGC TGG ATG AAG - 3'(SEQ ID NO:53)

The paragraph beginning at page 115, line 22 has been amended as follows:

The PCR amplified sequence is then purified by gel electrophoresis and used as template for dideoxy sequencing reactions using the following oligonucleotide as a primer:

5' - GAG GTA CAC TGA CAT ACT GGC ATG - 3'(SEQ ID NO:54)

The paragraph beginning at page 116, line 22 has been amended as follows:

Double-stranded cDNA is prepared and isolated as described herein before. The double-stranded cDNA is denatured and used as a template for a third round of DNA synthesis using the following oligonucleotide primer:

5' - GTA CGC CAT ATC AGC TGG ATG AAG ACA GGA GAC GAG GGG GAA AAG GGT TGG GGC GGA TGC - 3'(SEQ ID NO:55)

The paragraph beginning at page 116, line 30 has been amended as follows:

This primer contains sequences specific for the constant portion of the μ heavy chain message (ACA GGA GAC GAG GGG GAA AAG GGT TGG GGC GGA TGC) (SEQ ID NO:56) as well as unique sequences that can be used as a primer for the PCR amplification of the newly synthesized DNA strand (GTA CGC CAT ATC AGC TGG ATG AAG) (SEQ ID NO:53). The sequence is amplified by PCR using the following two oligonucleotide primers:

5' - GAG GTA CAC TGA CAT ACT GGC ATG - 3'(SEQ ID NO:54)
5' - GTA CTC CAT ATC AGC TGG ATG AAG - 3'(SEQ ID NO:57)

The paragraph beginning at page 117, line 1 has been amended as follows:

The PCR amplified sequence is then purified by gel electrophoresis and used as template for dideoxy sequencing reactions using the following oligonucleotides as a primer:

5' - GAG GTA CAC TGA CAT ACT GGC ATG - 3'(SEQ ID NO:54)

The paragraph beginning at page 122, line 35 has been amended as follows

Mouse kappa chain sequences (Fig. 20a) were isolated from a genomic phage library derived from liver DNA using oligonucleotide probes specific for the Cκ locus:

5' - GGC TGA TGC TGC ACC AAC TGT ATC CAT CTT CCC ACC ATC CAG - 3'(SEQ ID NO:58)

and for the Jκ5 gene segment:

5' - CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGT AAG - 3'(SEQ ID NO:59).

The paragraph beginning at page 117, line 1 has been amended as follows:

Mouse heavy chain sequences containing the J_H region (Fig. 21a) were isolated from a genomic phage library derived from the D3 ES cell line (Gossler et al., Proc. Natl. Acad. Sci. U.S.A. 83:9065-9069 (1986)) using a J_H4 specific oligonucleotide probe:

5' - ACT ATG CTA TGG ACT ACT GGG GTC AAG GAA CCT CAG TCA CCG - 3'(SEQ ID NO:60)

The paragraph beginning at page 128, line 10 has been amended as follows:

Further construction of the targeting vector was carried out in a derivative of the plasmid pGP1b. pGP1b was digested with the restriction enzyme NotI and ligated with the following oligonucleotide as an adaptor:

5'- GGC CGC TCG ACG ATA GCC TCG AGG CTA TAA ATC TAG AAG AAT TCC AGC AAA GCT TTG GC -3'(SEQ ID NO:61)

The paragraph beginning at page 132, line 5 has been amended as follows:

The plasmid pBR322 was digested with EcoRI and StyI and ligated with the following oligonucleotides:

oligo-42 5'- caa gag ccc gcc taa tga gcg ggc ttt ttt ttg cat act gcg gcc gct -3'(SEQ ID NO:62)

oligo-43 5'- att tag cgg ccg cag tat gca aaa aaa agc ccg ctc att agg cgg gct -3'(SEQ ID NO:63)

The paragraph beginning at page 132, line 42 has been amended as follows:

GCggccgcctcgagatcactatcgattaattaaggatccagcagtaagcttgcGGCCGC (SEQ ID NO:64)

The paragraph beginning at page 133, line 4 has been amended as follows:

GCggccgcatcccgggtctcgaggtcgacaagctttcgaggatccgcGGCCGC(SEQ ID NO:65)

The paragraph beginning at page 133, line 10 has been amended as follows:

GCggccgctgtcgacaagcttatcgatggatcctcgagtgcGGCCGC(SEQ ID NO:66)

The paragraph beginning at page 133, line 16 has been amended as follows:

GCggccgctgtcgacaagcttcgaattcagatcgatgtggtacctggatcctcgagtgcGGCCGC(SEQ ID NO:67)

The paragraph beginning at page 133, line 24 has been amended as follows:

pGP1a was digested with NotI and ligated with the following oligonucleotides:

oligo-47 5'- ggc cgc aag ctt act gct gga tcc tta att aat cga tag tga tct cga ggc -3'(SEQ ID NO:68)

oligo-48 5'- ggc cgc ctc gag atc act atc gat taa tta agg atc cag cag taa gct tgc -3'(SEQ ID NO:69)

The paragraph beginning at page 133, line 40 has been amended as follows:

The following oligonucleotides:

oligo-44 5'- ctc cag gat cca gat atc agt acc tga aac agg gct tgc -3'(SEQ ID NO:70)

oligo-45 5'- ctc gag cat gca cag gac ctg gag cac aca cag cct tcc -3'(SEQ ID NO:71)

The paragraph beginning at page 136, line 5 has been amended as follows:

A human placental genomic CAN library cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA) was screened with the human heavy chain J region specific oligonucleotide:

oligo-1 5'- gga ctg tgt ccc tgt gtg atg ctt ttg atg tct ggg gcc aag -3' (SEQ ID NO:73)

and the phage clone λ1.3 isolated. A 6 kb HindIII/KpnI fragment from this clone, containing all six J segments as well as D segment DHQ52 and the heavy chain J-μ intronic enhancer, was isolated. The same library was screened with the human μ specific oligonucleotide:

oligo-2 5'- cac caa gtt gac ctg cct ggt cac aga cct gac cac cta tga -3'(SEQ ID NO:74)

and the phage clone λ2.1 isolated. A 10.5 kb HindIII/XhoI fragment, containing the μ switch region and all of the a μ constant region exons, was isolated from this clone. These two fragments were ligated together with KpnI/XhoI digested pNNO3 to obtain the plasmid pJM1.

The paragraph beginning at page 137, line 6 has been amended as follows:

The following human D region specific oligonucleotide:

oligo-4 5'- tgg tat tac tat ggt tcg ggg agt tat tat aac cac agt gtc -3' (SEQ ID NO:75)

was used to screen the human placenta genomic library for D region clones. Phage clones λ4.1 and λ4.3 were isolated. A 5.5 kb XhoI fragment, that includes the D elements D_{K1}, D_{N1}, and D_{M2} (Ichihara et al., EMBO J. 7:4141 (1988)), was isolated from phage clone λ4.1. An adjacent upstream 5.2 kb XhoI fragment, that includes the D elements D_{LR1}, D_{XP1}, $D_{XP'1}$, and D_{A1}, was isolated from phage clone λ4.3. Each of these D region XhoI fragments were cloned into the SalI site of the plasmid vector pSP72 (Promega, Madison, WI) so as to destroy the XhoI site linking the two sequences. The upstream fragment was then excised with XhoI and SmaI, and the downstream fragment with EcoRV and XhoI. The resulting isolated fragments were ligated together with SalI digested pSP72 to give the plasmid pDH1. pDH1 contains a 10.6 kb insert that includes at least 7 D segments and can be excised with XhoI (5') and EcoRV (3').

The paragraph beginning at page 138, line 22 has been amended as follows:

The following oligonucleotide, specific for human Ig g constant region genes:

oligo-29 5'- cag cag gtg cac acc caa tgc cca tga gcc cag aca ctg gac -3'(SEQ ID NO:76)

was used to screen the human genomic library. Phage clones 129.4 and λ29.5 were isolated. A 4 kb HindIII fragment of phage clone λ29.4, containing a γ switch region, was used to probe a human placenta genomic DNA library cloned into the phage vector lambda FIX™ II (Stratagene, La Jolla, CA). Phage clone λSg1.13 was isolated. To determine the subclass of

the different γ clones, dideoxy sequencing reactions were carried out using subclones of each of the three phage clones as templates and the following oligonucleotide as a primer:

oligo-67 5'- tga gcc cag aca ctg gac -3' (SEQ ID NO:77)

The paragraph beginning at page 140, line 19 has been amended as follows:

The human placental genomic DNA library lambda, FIX™ II, Stratagene, La Jolla, CA) was screened with the following human VH1 family specific oligonucleotide:

oligo-49 5'- gtt aaa gag gat ttt att cac ccc tgt gtc ctc tcc aca ggt gtc -3'(SEQ ID NO:78)

Table 2 on page 141, has been replaced with the attached replacement Table 2.

The paragraph beginning at page 142, line 31 has been amended as follows:

The pSγ1-5' insert was excised with SmaI and HindIII, treated with Klenow enzyme, and ligated with the following oligonucleotide linker:

5'-ccg gtc gac cgg –3' (SEQ ID NO:81)

The paragraph beginning at page 146, line 30 has been amended as follows:

Polyadenylated RNA was isolated from an eleven week old male second generation line-57 pHC1 transgenic mouse. This RNA was used to synthesize oligo-dT primed single stranded cDNA. The resulting cDNA was then used as template for four individual PCR amplifications using the following four synthetic oligonucleotides as primers: VH251 specific oligo-149, cta gct cga gtc caa gga gtc tgt gcc gag gtg cag ctg (g,a,t,c) (SEQ ID NO:82); VH105 specific o-150, gtt gct cga gtg aaa ggt gtc cag tgt gag gtg cag ctg (g,a,t,c) (SEQ ID NO:83); human gamma1 specific oligo-151, ggc gct cga gtt cca cga cac cgt cac cgg ttc (SEQ ID NO:84); and human mu specific oligo-152, cct gct cga ggc agc caa cgg cca cgc tgc tcg (SEQ ID NO:85). Reaction 1 used primers 0-149 and o-151 to amplify VH251-gamma1 transcripts, reaction 2 used o-149 and o-152 to amplify VH251-mu transcripts, reaction 3 used o-150 and o-151 to amplify VH105-gamma1 transcripts, and reaction 4 used

o-150 and o-152 to amplify VH105-mu transcripts. The resulting 0.5 kb PCR products were isolated from an agarose gel; the μ transcript products were more abundant than the γ transcript products, consistent with the corresponding ELISA data (Fig. 34). The PCR products were digested with XhoI and cloned into the plasmid pNN03. Double-stranded plasmid DNA was isolated from minipreps of nine clones from each of the four PCR amplifications and dideoxy sequencing reactions were performed. Two of the clones turned out to be deletions containing no D or J segments. These could not have been derived from normal RNA splicing products and are likely to have originated from deletions introduced during PCR amplification. One of the DNA samples turned out to be a mixture of two individual clones, and three additional clones did not produce readable DNA sequence (presumably because the DNA samples were not clean enough). The DNA sequences of the VDJ joints from the remaining 30 clones are compiled in Table 4. Each of the sequences are unique, indicating that no single pathway of gene rearrangement, or single clone of transgene expressing B-cells is dominant. The fact that no two sequences are alike is also an indication of the large diversity of immunoglobulins that can be expressed from a compact minilocus containing only 2 V segments, 10 D segments, and 6 J segments. Both of the V segments, all six of the J segments, and 7 of the 10 D segments that are included in the transgene are used in VDJ joints. In addition, both constant region genes (mu and gamma1) are incorporated into transcripts. The VH105 primer turned out not to be specific for VH105 in the reactions performed. Therefore many of the clones from reactions 3 and 4 contained VH251 transcripts. Additionally, clones isolated from ligated reaction 3 PCR product turned out to encode IgM rather than IgG; however this may reflect contamination with PCR product from reaction 4 as the DNA was isolated on the same gel. An analogous experiment, in which immunoglobulin heavy chain sequences were amplified from adult human peripheral blood lymphocytes (PBL), and the DNA sequence of the VDJ joints determined, was recently reported by Yamada et al. (J. Exp. Med. 173:395-407 (1991)), which is incorporated herein by reference). we compared the data from human PBL with our data from the pHC1 transgenic mouse.

The paragraph beginning at page 149, line 1 has been amended as follows:

TABLE 4 (SEQ ID NOS:86-115)

The paragraph beginning at page 152, line 2 has been amended as follows:

TABLE 7 (SEQ ID NOS:116-145)

The paragraph beginning at page 153, line 32 has been amended as follows:

Two human leukocyte genomic DNA libraries cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA) are screened with a 1 kb PacI/HindIII fragment of λ1.3 containing the human heavy chain J- μ intronic enhancer. Positive clones are tested for hybridization with a mixture of the following V_H specific oligonucleotides:

oligo-7 5'- tca gtg aag gtt tcc tgc aag gca tct gga tac acc ttc acc-3' (SEQ ID NO:146)

oligo-8 5'- tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttc agt-3' (SEQ ID NO:147)

The paragraph beginning at page 154, line 26 has been amended as follows:

Plasmid vector pGP1a is digested with NotI and the following oligonucleotides ligated in:

oligo-81 5'- ggc cgc atc ccg ggt ctc gag gtc gac aag ctt tcg agg atc cgc-3' (SEQ ID NO:148)

oligo-82 5'- ggc cgc gga tcc tcg aaa gct tgt cga cct cga gac ccg gga tgc-3' (SEQ ID NO:149)

The resulting plasmid, pGP1c, contains a polylinker with XmaI, XhoI, SalI, HindIII, and BamHI restriction sites flanked by NotI sites.

The paragraph beginning at page 155, line 2 has been amended as follows:

Plasmid vector pGP1a is digested with NotI and the following oligonucleotides ligated in:

oligo-87 5'- ggc cgc tgt cga caa gct tat cga tgg atc ctc gag tgc -3' (SEQ ID NO:150)

oligo-88 5'- ggc cgc act cga gga tcc atc gat aag ctt gtc gac agc -3' (SEQ ID NO:151)

The resulting plasmid, pGP1d, contains a polylinker with SalI, HindIII, ClaI, BamHI, and XhoI restriction sites flanked by NotI sites.

The paragraph beginning at page 155, line 16 has been amended as follows:

A human placental genomic DNA library cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA) was screened with the human kappa light chain J region specific oligonucleotide:

oligo-36 5'- cac ctt cgg cca agg gac acg act gga gat taa acg taa gca -3'(SEQ ID NO:152)

and the phage clones 136.2 and 136.5 isolated. A 7.4 kb XhoI fragment that includes the $J_{\kappa}1$ segment was isolated from 136.2 and subcloned into the plasmid pNNO3 to generate the plasmid clone p36.2. A neighboring 13 kb XhoI fragment that includes Jκ segments 2 through 5 together with the Cκ gene segment was isolated from phage clone 136.5 and subcloned into the plasmid pNNO3 to generate the plasmid clone p36.5. Together these two clones span the region beginning 7.2 kb upstream of Jκ1 and ending 9 kb downstream of Cκ.

The paragraph beginning at page 157, line 1 has been amended as follows:

Two human leukocyte genomic DNA libraries cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA) were screened with the human kappa light chain J region containing 3.5 kb XhoI/SmaI fragment of p36.5. Positive clones were tested for hybridization with the following Vκ specific oligonucleotide:

oligo-65 5'- agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc-3'(SEQ ID NO:153)

Clones that hybridized with both V and J probes are isolated and the DNA sequence of the rearranged VJκ segment determined.

The paragraph beginning at page 159, line 37 has been amended as follows:

The NotI digested DNA is then fractionated by pulsed field gel electrophoresis as described by Anand et al., Nuc. Acids Res.. 17: 3425-3433 (1989). Fractions enriched for the NotI fragment are assayed by Southern hybridization to detect one or more of the sequences encoded by this fragment. Such sequences include the heavy chain D segments, J segments, and γ1 constant regions together with representatives of all 6 V_H families (although this fragment is identified as 670 kb fragment from HeLa cells by berman et al. (1988), supra., we have found it to be an 830 kb fragment from the human placental and sperm DNA. Those fractions containing this NotI fragment are ligated into the NotI cloning site of the vector pYACNN as described (McCormick et al., Technique 2:65-71 (1990)). Plasmid pYACNN is prepared by digestion of pYACneo (Clontech) with EcoRI and ligation in the presence of the oligonucleotide 5'– AAT TGC GGC CGC –3'(SEQ ID NO:25).

The paragraph beginning at page 165, line 10 has been amended as follows:

The vector pGP1b (referred to in a previous example) is digested with XhoI and BamHI and ligated with the following oligonucleotides:

5'- gat cct cga gac cag gta cca gat ctt gtg aat tcg -3'(SEQ ID NO:154)

5'- tcg acg aat tca caa gat ctg gta cct ggt ctc gag -3'(SEQ ID NO:155)

to generate the plasmid pGP1h. This plasmid contains a polylinker that includes the following restriction sites: NotI, EcoRI, BglII, Asp718, XhoI, BamHI, HindIII, NotI.

The paragraph beginning at page 166, line 22 has been amended as follows:

The following two oligonucleotides:

5'- cgc ggt acc gag agt cag tcc ttc cca aat gtc -3'(SEQ ID NO:156)

5'- cgc ctc gag aca gct gga atg ggc aca tgc aga -3'(SEQ ID NO:157)

are used as primers for the amplification of mouse IgM constant region sequences by polymerase chain reaction (PCR) using mouse spleen cDNA as a substrate. The resulting 0.3 kb PCR product is digested with Asp718 and XhoI and cloned into Asp718/XhoI digested pBCE1 to generate the antisense transgene construct pMAS1. The purified NotI insert of pMAS1 is microinjected into the pronuclei of half day mouse embryos--alone or in combination with one or more other transgene constructs--to generate transgenic mice. This construct expresses an RNA transcript in B-cells that hybridizes with mouse IgM mRNA, thus down-regulating the expression of mouse IgM protein. Double transgenic mice containing pMAS1 and a human heavy chain transgene minilocus such as pHC1 (generated either by coinjection of both constructs or by breeding of singly transgenic mice) will express the human transgene encoded Ig receptor on a higher percentage of B-cell than mice transgenic for the human heavy chain minilocus alone. The ratio of human to mouse Ig receptor expressing cells is due in part to competition between the two populations for factors and cells that promote[r] B-cell differentiation and expansion. Because the Ig receptor plays a key role in B-cell development, mouse Ig receptor expressing B-cells that express reduced levels of IgM on their surface (due to mouse Ig specific antisense down-regulation) during B-cell development will not compete as well as cells that express the human receptor.

The paragraph beginning at page 167, line 17 has been amended as follows:

The following two oligonucleotides:

5'- cgc ggt acc gct gat gct gca cca act gta tcc -3'(SEQ ID NO:158)

5'- cgc ctc gag cta aca ctc att cct gtt gaa gct -3'(SEQ ID NO:159)

are used as primers for the amplification of mouse IgKappa constant region sequences by polymerase chain reaction (PCR) using mouse spleen cDNA as a substrate. The resulting 0.3 kb PCR product is digested with Asp718 and XhoI and cloned into Asp718/XhoI digested pBCE1 to generate the antisense transgene construct pKAS1. The purified NotI insert of pKAS1 is microinjected into the pronuclei of half day mouse embryos--alone or in combination with one or more other transgene constructs--to generate transgenic mice. This

construct expresses an RNA transcript in B-cells that hybridizes with mouse IgK mRNA, thus down-regulating the expression of mouse IgK protein as described above for pMAS1.

The paragraph beginning at page 171, line 11 has been amended as follows:

Line HC1-57 transgenic mice, containing multiple copies of the HC1 transgene, were bred with immunoglobulin heavy chain deletion mice to obtain mice that contain the HC1 transgene and contain disruptions at both alleles of the endogenous mouse heavy chain (supra). These mice express human mu and gamma1 heavy chains together with mouse kappa and lambda light chains (supra). One of these mice was hyperimmunized against human carcinoembryonic antigen by repeated intraperitoneal injections over the course of 1.5 months. This mouse was sacrificed and lymphoid cells isolated from the spleen, inguinal and mesenteric lymph nodes, and [peyers] Peyer's patches. The cells were combined and total RNA isolated. First strand cDNA was synthesized from the RNA and used as a template for PCR amplification with the following 2 oligonucleotide primers:

149 5'- cta gct cga gtc caa gga gtc tgt gcc gag gtg cag ctg (g/a/t/c)-3'(SEQ ID NO:82)

151 5'- ggc gct cga gtt cca cga cac cgt cac cgg ttc-3'(SEQ ID NO:184)

The paragraph beginning at page 172, line 24 has been amended as follows:

The Vκ specific oligonucleotide, oligo-65 (5'- agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc-3')(SEQ ID NO:153), was used to probe a human placental genomic DNA library cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA). DNA fragments containing Vκ segments from positive phage clones were subcloned into plasmid vectors. Variable gene segments from the resulting clones are sequenced, and clones that appear functional were selected. Criteria for judging functionality include: open reading frames, intact splice acceptor and donor sequences, and intact recombination sequence. DNA sequences of 4 functional Vκ gene segments (vk65.3, vk65.5, vk65.8, and vk65.15) from 4 different plasmid clones isolated by

this procedure are shown in Figs. 41-44. The four plasmid clones, p65.3f, p65.5gl, p65.8, and p65.15f, are described below.

The paragraph beginning at page 177, line 20 has been amended as follows:

RNA was isolated from a hyperimmunized HC1 line 57 transgenic mouse homozygous for the endogenous heavy chain J segment deletion (*supra*). cDNA was synthesized according to Taylor et al. (1993) Nucleic Acids Res. 20:6287, incorporated herein by reference, and amplified by PCR using the following two primers:

o-149 (human V_{H251}):

5'- CTA GCT CGA GTC CAA GGA GTC TGT GCC GAG GTG CAG CTG (G,A,T,C)-3'(SEQ ID NO:82)

o-249 (mouse gamma):

5'- GGC GCT CGA GCT GGA CAG GG(A/C) TCC A(G/T)A GTT CCA-3'(SEQ ID NO:160)

The paragraph beginning at page 198, line 31 has been amended as follows:

A 3.4 kb XhoI/EcoRI fragment covering the mouse heavy chain J region and the μ intronic enhancer is subcloned into a plasmid vector that contains a neomycin resistance gene as well as a herpes thymidine kinase gene under the control of a phosphoglycerate kinase promoter (tk/neo cassette; Hasty et al., (1991) Nature 350: 243). This clone is then used as a substrate for generating 2 different PCR fragments using the following oligonucleotide primers:

o-A1 5'- cca cac tct gca tgc tgc aga agc ttt tct gra -3'(SEQ ID NO:161)

o-A2 5'- ggt gac tga ggt acc ttg acc cca gta gtc cag-3'(SEQ ID NO:162)

o-A3 5'- ggt tac ctc agt cac cgt ctc ctc aga ggt aag aat ggc ctc -3'(SEQ ID NO:163)

o-A4 5'- agg ctc cac cag acc tct cta gac agc aac tac -3'(SEQ ID NO:164)

The paragraph beginning at page 199, line 31 has been amended as follows:

The resulting mouse contains a JH4 segment that has been converted from the unmutated sequence:

. .

.TGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAG___gtaagaatggcctctcc. . . (SEQ ID NO:165)

TrpBlyClnGlyThrSerValThrVA1SerSerGlu (SEQ ID NO:166)

to the mutant sequence:

. . .

TGGGGTCAAGGTACCTCAGTCACCGTCTCCTCAGAGgtaagaatggcctctcc. . . (SEQ ID NO:167)

TrpGlyGlnGlyThrSerValThrV[A]alSerSerGlu (SEQ ID NO:168)

The sequence of the unmutated μ gene is:

. . . ctggtcctcagAGAGTCAGTCCTTCCCAAATGTCTTCCCCCTCGTC. . .

GluSerGlnSerPheProAsnValPheProLeuVal

The paragraph beginning at page 200, line 9 has been amended as follows:

The sequence of the unmutated μ gene is:

XmnI

. . . ctggtcctcagAGAGTCAGTCCTTCCCAAATGTCTTCCCCTCGTC. . .(SEQ ID NO:169)

GluSerGlnSerPheProAsnValPheProLeuVal (SEQ ID NO:170)

The sequence of the mutated μ gene is:

XmnI

. . . ctggtcctcag___AGTCAGTCCTTCCCGAATGTCTTCCCCTCGTC. . .(SEQ ID NO:171)

SerGlnSerPheProAsnValPheProLeuVal (SEQ ID NO:172)

The homologous recombination vector containing the mutant sequence is linearized and electroporated into an ES cell line containing the JH4 insertion. Homologous recombinants are identified from neomycin-resistant clones. Those homologous recombinants that contain the frameshift insertion on the same chromosome as the JH4 insertion are identified by Southern blot hybridization of KpnI/BamHI digested DNA. The JH4 insertion is associated with a new KpnI site that reduces the size of the J- μ intron containing KpnI/BamHI fragment from the wild type 11.3 kb to a mutant 9 kb. The resulting clones are then selected for deletion of the inserted tk/neo cassette using FIAU. Clones containing the mutant μ exon are identified by Southern blot hybridization of XmnI digested DNA. The mutation is confirmed by DNA sequence analysis of PCR amplified μ exon1 DNA.

The paragraph beginning at page 213, line 18 has been amended as follows:

The human light chain transgene, KCo4, depicted in Fig. 56 is generated by the cointegration of two individually cloned DNA fragments at a single site in the mouse genome. The fragments comprise 4 functional Vκ segments, 5J segments, the C κ exon, and both the intronic and downstream enhancer elements (see Example 21) (Meyer and Neuberger (1989), EMBO J. 8:1959-1964; Judde and Max (1992), Mol. Cell Biol. 12: 5206-5216). Because the two fragments share a common 3 kb sequence (see Fig. 56), they can potentially integrate into genomic DNA as a contiguous 43 kb transgene, following homologous recombination between the overlapping sequences. It has been demonstrated that such recombination events frequently occur upon microinjection of overlapping DNA fragments (Pieper et al., (1992), Nucleic Acids Res. 20:1259-1264). Co-injected DNA[']s also tend to co-integrate in the zygote, and the sequences contained within the individually cloned fragments would subsequently be jointed by DNA rearrangement during B cell development. Table [12] 11 shows that transgene inserts from at least 2 of the transgenic lines are functional. Examples of VJ junctions incorporating each of the 4 transgene encoded V segments, and each of the 5J segments, are represented in this set of 36 clones.

The paragraph beginning at page 215, line 1 has been amended as follows:

Table [12] 11

The paragraph beginning at page 215, line 5 has been amended as follows:

Human light chain V and J segment usage in KCo4 transgenic mice. The table shows the number of PCR clones, amplified from cDNA derived from two transgenic lines, which contain the indicated human kappa sequences. cDNA was synthesized using spleen RNA isolated from w individual KCo4 transgenic mice (mouse #8490, 3 mo., male, KCo4 line 4437; mouse #8867, 2.5 mo., female, KCo4 line 4436). The cDNA was amplified by PCR using a C κ specific oligonucleotide, 5' TAG AAG GAA TTC AGC AGG CAC ACA ACA GAG GCA GTT CCA 3'(SEQ ID NO:173), [AND A] and a 1:3 mixture of the following 2 V κ specific oligonucleotides: 5'AGC TTC TCG AGC TCC TGC TGC TCT GTT TCC CAG GTG CC 3'(SEQ ID NO:174) and 5' CAG CTT CTC GAG CTC CTG CTA CTC TGG CTC (C,A)CA GAT ACC 3'(SEQ ID NO:175). The PCR product was digested with XhoI and EcoRI, and cloned into a plasmid vector. Partial nucleotide sequences were determined by the dideoxy chain termination method for 18 randomly picked clones from each animal. The sequences of each clone were compared to the germline sequence of the unrearranged transgene.

The paragraph beginning at page 216, line 10 has been amended as follows:

Table [13] 12 shows that somatic mutation occurs in the variable regions of the transgene-encoded human heavy chain transcripts of the transgenic mice. Twenty-three cDNA clones from a HC2 transgenic mouse were partially sequenced to determine the frequency of non-germline encoded nucleotides within the variable region. The data include only the sequence of V segment codons 17-94 from each clone, and does not include N regions. RNA was isolated from the spleen and lymph node of mouse 5250 (HC2 line 2550 hemizygous, JHD homozygous). Single-stranded cDNA was synthesized and γ transcripts amplified by PCR as described [references]. The amplified cDNA was cloned into plasmid vectors, and 23 randomly picked clones were partially sequenced by the dideoxy chain-

termination method. The frequency of PCR-introduced nucleotide changes is estimated from constant region sequence as <0.2%.

The paragraph beginning at page 216, line 27 has been amended as follows:

TABLE [13] **12**: The Variable Regions of Human γ Transcripts in HC2 Transgenic Mice Contain Non-Germline-Encoded Nucleotides

The paragraph beginning at page 225, line 18 has been amended as follows:

The characteristics of the three hybridomas, 2Cll.8, 2C5.1, and 4e4.2, are given below in Table [11]13.

The paragraph beginning at page 225, line 21 has been amended as follows:

Table [11]**13** Human variable region usage in hybridomas

The paragraph beginning at page 225, line 38 has been amended as follows:

VH251	N	DHQ52	JH5
TAT TAC TCT CCC AC	'g ggt gg'	A ACT GGG GA	C TGG TTC GAC (SEQ ID NO:176)
Y Y C A R	A P	T G C	W F D (SEQ ID NO:177)

The paragraph beginning at page 226, line 3 has been amended as follows:

The light chain VJ junction is:

Vk65.15	N	Jk4
TAT AAT AGT TAC CCT CC	(t)	ACT TTC GGC (SEQ ID NO:178)
Y N S Y P P		T F G (SEQ ID NO:179)

The paragraph beginning at page 226, line 3 has been amended as follows:

We conclude that these two gamma hybridomas are derived from B cells that have undergone a limited amount of somatic mutation. This data shows that the HC2 transgenic animals use the VH5-51 (aka VH251) V segment. We have previously shown that VH4-34, VH1-69, and VH3-30.0 are expressed by these mice. The combination of these results demonstrates that the HC2 transgenic [@ce] mice ecpress all four of the transgene encoded human VH genes.

The paragraph beginning at page 227, line 16 has been amended as follows:

A set of five different plasmid clones was constructed such that the plasmid inserts could be isolated , substantially free of vector sequences; and such that the inserts together form a single imbricate set of overlapping sequence spanning approximately 150 kb in length. This set includes human V, D, J, μ, γ3, and γ1 coding sequences, as well as a mouse heavy chain 3' enhancer sequence. The five clones are, in 5' to 3' order: pH3V4D, pCOR1xa, p11-14, pP1-570, and pHP-3a (Fig. 76). Several different cloning vectors were used to generate this set of clones. Some of the vectors were designed specifically for the purpose of building large transgenes. These vectors (pGP1a, pGP1b, pGP1c, pGP1d, pGP1f, pGP2a, and pGP2b) are PBR322-based plasmids that are maintained at a lower copy number per cell than the pUC vectors (Yanisch-Verron et al. (1985) Gene 33: 103-119). The vectors also include trpA transcription termination signals between the polylinker and the 3' end of the β-lactamase gene. The polylinkers are flanked by restriction sites for the rare-cutting enzyme NotI; thus allowing for the isolation of the insert away from vector sequences prior to embryo microinjection. Inside of the NotI sites, the polylinkers include unique XhoI and SalI sites at either end. The pGP1 vectors are described in Taylor et al. (1992) Nucleic Acids Res. 23: 6287. To generate the pGP2 vectors, pGP1f was first digested with AlwNI and ligated with the synthetic oligonucleotides o-236 and o-237 (o-236, 5'- ggc gcg cct tgg cct aag agg cca -3'; o-237(SEQ ID NO:180), 5'- cct ctt agg cca agg cgc gcc tgg -3'(SEQ ID NO:181)) The resulting plasmid is called pGP2a. Plasmid pGP2a was then digested with KpnI and EcoRI, and ligated with the oligonucleotides o-288 and o-289 (o-288, 5'- aat tca gta tcg atg

tgg tac-3'(SEQ ID NO:182); o-289, 5'- cac atc gat act g-3'(SEQ ID NO:183)) to create pGP2b (Figs. 77A and Fig. 77B).

The paragraph beginning at page 229, line 23 has been amended as follows:

Germline configuration heavy chain variable gene segments were isolated from phage 1 genomic DNA libraries using synthetic oligonucleotide probes for VH1 and VH3 classes. The VH1 class probe was o-49:

5'- gtt aaa gag gat ttt att cac ccc tgt gtc ctc tcc aca ggt gtc -3'(SEQ ID NO:78)

The VH3 class probe was o-184:

5'- gtt tgc agg tgt cca gtg t(c,g)a ggt gca gct g(g,t)t gga gtc (t,c)(g,c)g-3'(SEQ ID NO:184)

The paragraph beginning at page 231, line 10 has been amended as follows:

The 10.6 kb XhoI/EcoRV insert of the human D region clone pDH1 (described supra; e.g., in Example 12) was cloned into XhoI/EcoRV digested pGPe plasmid vector to create the new plasmid pDH1e. This plasmid was then digested with EcoRV and ligated with a synthetic linker fragment containing a SalI site (5'- ccg gtc gac ccg -3') (SEQ ID NO:185). The resulting plasmid, pDH1es, includes most of the human D1 cluster within an insert that can be excised with XhoI and SalI, such that the XhoI site is on the 5' end, and the SalI site is on the 3' end. This insert was isolated and cloned into the SalI site of pH3VH4 to create the plasmid Ph3VH4D, which includes four germline configuration human VH gene segments and 8 germline configuration human D segments, all in the same 5' to 3' orientation. The insert of this clone can be isolated, substantially free of vector sequences, by digestion with NotI.

The paragraph beginning at page 232, line 1 has been amended as follows:

A phage P1 library (Genome Systems Inc., St. Louis, Missouri) was screened by PCR using the oligonucleotide primer pair:

5'- tca caa gcc cag caa cac caa g-3'(SEQ ID NO:186)

5'- aaa agc cag aag acc ctc tcc ctg -3'(SEQ ID NO:187)

This primer pair was designed to generate a 216 bp PCR product with a human γ gene template. One of the P1 clones identified was found to contain both the human γ3 and γ1 genes within an 80 kb insert. The insert of this clone, which is depicted in Fig. 80, can be isolated, substantially free of vector sequences, by digestion with NotI and SalI.

The paragraph beginning at page 232, line 34 has been amended as follows:

The mouse heavy chain 3' enhancer (Dariavach et al. (1991) Eur. J. Immunol. 21: 1499; Lieberson et al. (1991) Nucleic Acids Res. 19: 933) was cloned from balb/c mouse genomic DNA phage λ library. To obtain a probe, total balb/c mouse thymus DNA was used as a template for PCR amplification using the following two oligonucleotides:

cck76: 5'- caa tag ggg tca tgg acc c-3'(SEQ ID NO:188)

cck77: 5'- tca ttc tgt gca gag ttg gc -3'(SEQ ID NO:189)

The paragraph beginning at page 234, line 22 has been amended as follows:

Tail tip DNA was isolated from 202 animals that developed from the microinjected embryos. Southern blot analysis of this DNA, using a probe comprising human μ and DH sequences, revealed 22 founder animals that had incorporated at least a portion of the HCo7 transgene. Fig. 81 shows an analysis of the expression of huamn μ and γ1 in the serum of 6 G0 animals that had developed from embryos microinjected with HCo& DNA. Serum levels of human immunoglobulin proteins were measured by ELISA as described in Lonberg et al. (1994) Nature 368: 856. Four of these six mice showed evidence of incorporation of the transgene by Southern blot analysis, and three of these mice expressed both human μ and human γ1 proteins in their serum. The single transgenic mouse that did not express human immunoglobulin proteins was determined by Southern blot analysis to contain only a low number of copies of the transgene, and it is possible that the entire transgene was not incorporated, or that this mouse was a genetic mosaic. Two of the founder

HCo7 mice #11952 and #11959, were bred with human κ minilocus (KCo4 line 4436) transgenic mice that were also homozygous for disruptions of the endogenous heavy , and k light chain loci (Lonberg et al. *op. cit.*), to generate mice that were homozygous for the two introduced human miniloci, KCo4 and HCo7. Five of these so-called double-transgenic/double-deletion mice were analyzed for expression of human IgM, human IgG1, and human IgG3. As a control, three HC2/KCo4 double-transgenic/double-deletion mice were included in the analysis. This experiment is presented in Fig. 82. The ELISA data in this figure was collected as in Lonberg et al. (*op. cit*), except that for detection of human IgG3, the coating antibody was a specific mAb directed against human IgG3 (cat. # 08041, Pharmingen, La Jolla, CA); the other details of the IgG3 assay were identical to those published for IgG1. While the HC2/KCo4 mice express only human IgM and human IgG1, the HCo7/KCo4 mice also express human IgG3 in addition to these two isotypes. Expression of human g3 and g1 in the HCo7 mice has also been detected by PCR amplification of cDNA synthesized from RNA isolated from the spleen of a transgenic mouse. Fig. 83 depicts PCR amplification products synthesized using spleen cDNA from three different lines of transgenic mice: line 2550 is an HC2 transgenic line, while lines 11959 and 11952 are HCo7 transgenic lines. Single stranded cDNA was synthesized from spleen RNA as described by Taylor et al. (1992) Nucleic Acid Res. 20: 6287. The cDNA was then PCR amplified using the following two oligonucleotides:

o-382: 5'- gtc cag aat tcg gt(c,g,t) cag ctg gtg (c,g)ag tct gg-3'(SEQ ID NO:190)

o-383: 5'- ggt ttc tcg agg aag agg aag act gac ggt cc-3'(SEQ ID NO:191)

The paragraph beginning at page 237, line 15 has been amended as follows:

Total genomic DNA was isolated from a yeast strain containing a 450 kb yeast artificial chromosome (YAC) comprising a portion of the human Vκ locus (ICRF YAC library designation 4x17E1). To determine the identity of some of the Vκ gene segments

included in this YAC clone, the genomic DNA was used as a substrate for a series of Vκ family specific PCR amplification reactions. Four different 5' primers were each paired with a single consensus 3' primer in four sets of amplifications. The 5' primers were: o-270 (5'-gac atc cag ctg acc cag tct cc-3'(SEQ ID NO:192)), o-271 (5'-gat att cag ctg act cag tct cc-3'(SEQ ID NO:193), o-272 (5'-gaa att cag ctg acg cag tct cc-3'(SEQ ID NO:194)), and o-273 (5'-gaa acg cag ctg acg cag tct cc-3'(SEQ ID NO:195)). These primers are used by Marks et al. (Eur. J. Immunol. 1991. 21, 985) as Vκ family specific primers. The 3' primer, o-274 (5'-gca agc ttc tgt ccc aga ccc act gcc act gaa cc-3'(SEQ ID NO:196)), is based on a consensus sequence for FR3. Each of the four sets of primers directed the amplification of the expected 0.2 kb fragment from yeast genomic DNA containing the YAC clone 4x17E1. The 4 different sets of amplification products were then gel purified and cloned into the PvuII/HindIII site of the plasmid vector pSP72 (Promega). Nucleotide sequence analysis of 11 resulting clones identified seven distinct V genes. These results are presented below in Table 14.

The paragraph beginning at page 240, line 12 has been amended as follows:

A Southern blot analysis of genomic DNA from mice of lines KCo5-9269 and KCo5-9272 was carried out to determine if YAC 4x17E1 derived Vκ segments had been incorporated in their genomes. A V_K gene segment, VkA10 (accession #: x12683; Straubinger et al. 1988. Biol. Chem. Hoppe-Seyler 369, 601-607), from the middle of the distal Vκ cluster was chosen as a probe for the Southern Blot analysis. To obtain the cloned probe, the VkA10 gene was first amplified by PCR. The two oligo nucleotides, o-337 (5'-cgg tta aca tag ccc tgg gac gag ac-3'(SEQ ID NO:197)) and o-338 (5'- ggg tta act cat tgc ctc caa agc ac-3'(SEQ ID NO:198)), were used as primers to amplify a 1 kb fragment from YAC 4x17E1. The amplification product was gel purified, digested with HincII, and cloned into pUC18 to obtain the plasmid p17E1A10. The insert of this plasmid was then used to probe a southern blot of KCo5-9269 and KCo5-9272 DNA. The blot showed hybridization of the probe to the expected restriction fragments in the KCo5-9272 mouse DNA only. This

indicates that the VkA10 gene is incorporated into the genome of KCo5-9272 mice and not KCo5-9269 mice. Line KCo5-9272 mice were then bred with HC2-2550/JHD/JKD mice to obtain mice homozygous for disruptions of the endogenous heavy and $_K$ light chain loci, and hemi- or homozygous for the HC2 and KCo5 transgenes. Animals that are homozygous for disruptions of the endogenous heavy and κ light chain loci, and hemi-or homozygous for human heavy and κ light chain transgenes are designated double transgenic/double deletion mice.

The paragraph beginning at page 241, line 1 has been amended as follows:

A cDNA cloning experiment was carried out to determine if any of the YAC-derived Vκ genes are expressed in line KCo5-9272 mice. The double transgenic/double deletion mouse #12648 (HC2-2550/KCo5-9272/JHD/JKD) was sacrificed and total RNA isolated from the spleen. Single stranded cDNA was synthesized from the RNA and used as a template in four separate PCR reactions using oligonucleotides o-270, o-271, o-272, and o-273 as 5' primers, and the Ck specific oligonucleotide, o-186 (5'-tag aag gaa ttc agc agg cac aca aca gag gca gtt cca -3'(SEQ ID NO:173)), as a 3' primer. The amplification products were cloned into the pCRII TA cloning vector (Invitrogen). The nucleotide sequence of 19 inserts was determined. The results of the sequence analysis are summarized in Table 15 below.

The paragraph beginning at page 256, line 5 has been amended as follows:

Cells from five individual hybridoma cell lines (1E11, 1G2, 6G5, 10C5, and 4D1) that secrete human IgG kappa monoclonal antibodies reactive with human CD4, and derived from JHD/JCKD/HC2/KCo5 transgenic mice, were used to isolate RNA encoding each of the individual antibodies (Fishwild et al. 1996, *Nature Biotechnology* 14, 845-851). The RNA was used as a substrate to synthesize cDNA, which was then used to amplify human Ig gamma and kappa transcript sequences by PCR using primers specific for human VH, Vkappa, Cgamma, and Ckappa (Taylor et al. 1992, *Nucleic Acids Res.* 20, 6287-6295;

Larrick, J.W., et al. (1989), *Bio/Technology.* 7. 934-938; Marks, J.D., et al. (1991). *Eur. J. Immunol.* 21. 985-991; Taylor, et al., 1994, *Int. Immunol.* 6, 579-591). The amplified Ig heavy and kappa light chain sequences were cloned into bacterial plasmids and nucleotide sequences determined. Analysis of the sequences spanning the heavy chain VDJ and light chain VJ junctions revealed in-frame heavy and light chain transcripts for each of the 5 clones, and in some cases additional out-of-frame sterile transcripts representing non-functional alleles. Consistent with proper functioning allelic exclusion, in no case was there more than one unique functional heavy or light chain transcript identified for each of the individual clones. Partial nucleotide sequences for each of the ten functional transcripts are assigned the following sequence I.D. No's: 1E11 gamma [Seq. I.D. No. __](SEQ ID NO:199); 1E11 kappa [Seq. I.D. No. __](SEQ ID NO:200); IG2 gamma [Seq. I.D. No. __](SEQ ID NO:201); 1G2 kappa [Seq. I.D. No. __](SEQ ID NO:202); 6G5 gamma [Seq. I.D. No. __](SEQ ID NO:203); 6G5 kappa [Seq. I.D. No. __](SEQ ID NO:204); 10C5 gamma [Seq. I.D. No. __](SEQ ID NO:205); 10C5 kappa [Seq. I.D. No. __](SEQ ID NO:206); 4D1 gamma [Seq. I.D. No. __](SEQ ID NO:207); 4D1 kappa [Seq. I.D. No. __](SEQ ID NO:208) and are presented in Table 22. All sequences are presented in a 5' to 3' orientation.

The paragraph beginning at page 257, line 6 has been amended as follows:

1E11 gamma [Seq. I.D. No. __](SEQ ID NO:199)

The paragraph beginning at page 257, line 66 has been amended as follows:

1E11 kappa [Seq. I.D. No. __](SEQ ID NO:200)

The paragraph beginning at page 257, line 21 has been amended as follows:

1G2 gamma [Seq. I.D. No. __](SEQ ID NO:201)

The paragraph beginning at page 257, line 31 has been amended as follows:

1G2 kappa [Seq. I.D. No. __](SEQ ID NO:202)

The paragraph beginning at page 257, line 36 has been amended as follows:

6G5 gamma [Seq. I.D. No. __](SEQ ID NO:203)

The paragraph beginning at page 258, line 8 has been amended as follows:

6G5 kappa [Seq. I.D. No. __](SEQ ID NO:204)

The paragraph beginning at page 258, line 13 has been amended as follows:

10C5 gamma [Seq. I.D. No. __](SEQ ID NO:205)

The paragraph beginning at page 258, line 23 has been amended as follows:

10C5 kappa [Seq. I.D. No. __](SEQ ID NO:206)

The paragraph beginning at page 258, line 32 has been amended as follows:

4D1 gamma [Seq. I.D. No. __](SEQ ID NO:207)

The paragraph beginning at page 259, line 5 has been amended as follows:

4D1 kappa [Seq. I.D. No. __](SEQ ID NO:208)

Table 23, beginning at page 260, line 1 has been amended as follows:

Table 23. Germline V(D)J Segment Usage in Hybridoma Transcripts

clone	h.c.CDR3	VH	DH	JH	1.c. CDR3	Vk	Jk
1E11	DDITMVRGVPH (Seq. I.D. No. [__]209)	VH4-34	DXP'1	JH4	QQYGSSPLT (Seq. I.D. No. 210[__])	VkA27/A1	Jk4
IG2	PPANWNWYFV L (Seq. I.D. No. [__]211)	VH5-51	DHQ52	JH2	QQFISYPQLT (Seq. I.D. No. [__]212)	VkL18	Jk4
6G5	VVINWFDP (Seq. I.D. No. [__]213)	VH4-34	n.d.	JH5	QQANSFPYT (Seq. I.D. No. [__]214)	VkL19	Jk2
10C5	VVINWFDP (Seq. I.D. No.213)	VH4-34	n.d.	JH5	QQANSFPYT (SEQ ID NO:214)	VkL19	Jk2
	DDQLGLFDY				QQYDSYPYT	VkL15	Jk2

4D1	(Seq. I.D. No. [__]215)	VH5-51	DHQ52	JH4	(Seq. I.D. No. [__]216)		

The paragraph beginning at page 260, line 26 has been amended as follows:

The kappa light chain plasmid, pCK7-96, includes the kappa constant region and polyadenylation site [[Seq. I.D. No. __]](SEQ ID NO:217), such that kappa sequences amplified with 5' primers that include HindIII sites upstream of the initiator methionine can be digested with Hind III and BbsI, and cloned into pCK7-96 digested with HindIII and BbsI to reconstruct a complete light chain coding sequence together with a polyadenylation site. This cassette can be isolated as a HindIII/NotI fragment and ligated to transcription promoter sequences to create a functional minigene for transfection into cells.

The paragraph beginning at page 261, line 7 has been amended as follows:

The gamma1 heavy chain plasmid, pCG7-96, includes the human gamma1 constant region and polyadenylation site [[Seq. I.D. No. __]](SEQ ID NO:218), such that gamma sequences amplified with 5' primers that include HindIII sites upstream of the initiator methionine can be digested with HindIII and AgeI, and cloned into pCG7-96 digested with HindIII and AgeI to reconstruct a complete gamma1 heavy chain coding sequence together with a polyadenylation site. This cassette can be isolated as a HindIII/SalI fragment and ligated to transcription promoter sequences to create a functional minigene for transfection into cells.

The paragraph beginning at page 261, line 19 has been amended as follows:

The following example demonstrates how nucleotide sequence data from hybridomas can be used to reconstruct functional Ig heavy and light chain minigenes. The nucleotide sequences of heavy and light chain transcripts from hybridomas 6G5 and 10C5 were used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa light chain sequences (designated HC6G5 [Seq. I.D. No. __](SEQ

ID NO:219) and LC6G5 [[Seq. I.D. No. __]](SEQ ID NO:220) differed from the natural sequences in three ways: strings of repeated nucleotide bases were interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites were incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266, 19867-19870); and, HindIII sites were engineered upstream of the translation initiation sites.

The paragraph beginning at page 262, line 4 has been amended as follows:

The following oligonucleotides were pooled: o=548 [[Seq. I.D. No. __]](SEQ ID NO:221), o-549 [[Seq. I.D. No. __]](SEQ ID NO:222), o-550 [[Seq. I.D. No. __]](SEQ ID NO:223), o-551 [[Seq. I.D. No. __]](SEQ ID NO:224), o-552 [[Seq. I.D. No. __]](SEQ ID NO:225), o-563 [[Seq. I.D. No. __]](SEQ ID NO:226), o-564 [[Seq. I.D. No. __]](SEQ ID NO:227), o-565 [[Seq. I.D. No. __]](SEQ ID NO:228), o-566 [[Seq. I.D. No. __]](SEQ ID NO:229), o-567 [[Seq. I.D. No. __]](SEQ ID NO:230), and amplified with the following primers: o-527 [[Seq. I.D. No. __]](SEQ ID NO:231) and o-562 [[Seq. I.D. No. __]](SEQ ID NO:232).

The paragraph beginning at page 262, line 14 has been amended as follows:

The following oligonucleotides were pooled: o-553 [[Seq. I.D. No. __]](SEQ ID NO:233), o-554 [[Seq. I.D. No. __]](SEQ ID NO:234), o-555 [[Seq. I.D. No. __]](SEQ ID NO:235), o-556 [[Seq. I.D. No. __]](SEQ ID NO:236), o-557 [[Seq. I.D. No. __]](SEQ ID NO:237), o-558 [[Seq. I.D. No. __]](SEQ ID NO:238), o-559 [[Seq. I.D. No. __]](SEQ ID NO:239), o-560 [[Seq. I.D. No. __]](SEQ ID NO:240), o-561 [[Seq. I.D. No. __]](SEQ ID NO:241), o-562 [[Seq. I.D. No. __]](SEQ ID NO:232), and amplified with the following 2 primers: o-552 [[Seq. I.D. No. __]](SEQ ID NO:225) and o-493 [[Seq. I.D. No. __]](SEQ ID NO:242).

The paragraph beginning at page 262, line 24 has been amended as follows:

The products of light chain PCR reactions 1 and 2 were then combined and amplified with the following two primers: o-493 [[Seq. I.D. No. __]](SEQ ID NO:242) and o-527 [[Seq. I.D. No. __]](SEQ ID NO:231).

The paragraph beginning at page 262, line 24 has been amended as follows:

The product of light chain PCR reaction 3 was then digested with HindIII and BbsI and cloned into HindIII/BbsI digested pCK7-96 [[Seq. I.D. No. __]](SEQ ID NO:217) to generate pLC6G5 [[Seq. I.D. No. __]](SEQ ID NO:243).

The paragraph beginning at page 262, line 36 has been amended as follows:

The following oligonucleotides were pooled: o-528 [[Seq. I.D. No. __]](SEQ ID NO:244), o-529 [[Seq. I.D. No. __]](SEQ ID NO:245), o-530 [[Seq. I.D. No. __]](SEQ ID NO:246), o-531 [[Seq. I.D. No. __]](SEQ ID NO:247), o-532 [[Seq. I.D. No. __]](SEQ ID NO:248), o-543 [[Seq. I.D. No. __]](SEQ ID NO:249), o-544 [[Seq. I.D. No. __]](SEQ ID NO:250), o-545 [[Seq. I.D. No. __]](SEQ ID NO:251), o-546 [[Seq. I.D. No. __]](SEQ ID NO:252), o-547 [[Seq. I.D. No. __]](SEQ ID NO:253), and amplified with the following 2 primers: o-496 [[Seq. I.D. No. __]](SEQ ID NO:254) and o-542 [[Seq. I.D. No. __]](SEQ ID NO:255).

The paragraph beginning at page 263, line 8 has been amended as follows:

The following oligonucleotides were pooled: o-533 [[Seq. I.D. No. __]](SEQ ID NO:256), o-534 [[Seq. I.D. No. __]](SEQ ID NO:257), o-535 [[Seq. I.D. No. __]](SEQ ID NO:258), o-536 [[Seq. I.D. No. __]](SEQ ID NO:259), o-537 [[Seq. I.D. No. __]](SEQ ID NO:260), o-538 [[Seq. I.D. No. __]](SEQ ID NO:261), o-539 [[Seq. I.D. No. __]](SEQ ID NO:262), o-540 [[Seq. I.D. No. __]](SEQ ID NO:263), o-541 [[Seq. I.D. No. __]](SEQ ID NO:264), o-542 [[Seq. I.D. No. __]](SEQ ID NO:255), together with the isolated 439 bp BbsI fragment of pCG7-96 [[Seq. I.D. No. __]](SEQ ID NO:218) and amplified with the following

2 primers: o-490 [[Seq. I.D. No. __]](SEQ ID NO:265) and o-520 [[Seq. I.D. No. __]](SEQ ID NO:266).

The paragraph beginning at page 263, line 19 has been amended as follows:

The products of heavy chain reactions 1 and 2 were then combined and amplified with the following two primers: o-520 [[Seq. I.D. No. __]](SEQ ID NO:266) and o-521 [[Seq. I.D. No. __]](SEQ ID NO:267).

The paragraph beginning at page 263, line 23 has been amended as follows:

The product of heavy chain reaction 3 was then digested with HindIII and AgeI and cloned into HindIII/AgeI digested pCG7-96 [[Seq. I.D. No. __]](SEQ ID NO:218) to generate pHC6G5 [[Seq. I.D. No. __]](SEQ ID NO:268).

The paragraph beginning at page 265, line 26 has been amended as follows:

pCK7-96 [[Seq. I.D. No. __]](SEQ ID NO:218)

The paragraph beginning at page 267, line 35 has been amended as follows:

O-548 [[Seq. I.D. No. __]](SEQ ID NO:221)

The paragraph beginning at page 268, line 1 has been amended as follows:

O-549 [[Seq. I.D. No. __]](SEQ ID NO:222)

The paragraph beginning at page 268, line 4 has been amended as follows:

O-550 [[Seq. I.D. No. __]](SEQ ID NO:223)

The paragraph beginning at page 268, line 7 has been amended as follows:

O-551 [[Seq. I.D. No. __]](SEQ ID NO:224)

The paragraph beginning at page 268, line 10 has been amended as follows:

O-552 [[Seq. I.D. No. __]](SEQ ID NO:225)

The paragraph beginning at page 268, line 13 has been amended as follows:

O-563 [[Seq. I.D. No. __]](SEQ ID NO:226)

The paragraph beginning at page 268, line 16 has been amended as follows:

O-564 [[Seq. I.D. No. __]](SEQ ID NO:227)

The paragraph beginning at page 268, line 19 has been amended as follows:

O-565 [[Seq. I.D. No. __]](SEQ ID NO:228)

The paragraph beginning at page 268, line 22 has been amended as follows:

O-566 [[Seq. I.D. No. __]](SEQ ID NO:229)

The paragraph beginning at page 268, line 25 has been amended as follows:

O-567 [[Seq. I.D. No. __]](SEQ ID NO:230)

The paragraph beginning at page 268, line 28 has been amended as follows:

O-527 [[Seq. I.D. No. __]](SEQ ID NO:231)

The paragraph beginning at page 268, line 31 has been amended as follows:

O-562 [[Seq. I.D. No. __]](SEQ ID NO:232)

The paragraph beginning at page 268, line 34 has been amended as follows:

O-553 [[Seq. I.D. No. __]](SEQ ID NO:233)

The paragraph beginning at page 268, line 37 has been amended as follows:

O-554 [[Seq. I.D. No. __]](SEQ ID NO:234)

The paragraph beginning at page 269, line 1 has been amended as follows:

O-555 [[Seq. I.D. No. __]](SEQ ID NO:235)

The paragraph beginning at page 269, line 4 has been amended as follows:

O-556 [[Seq. I.D. No. __]](SEQ ID NO:236)

The paragraph beginning at page 269, line 7 has been amended as follows:

O-557 [[Seq. I.D. No. __]](SEQ ID NO:237)

The paragraph beginning at page 269, line 10 has been amended as follows:

O-558 [[Seq. I.D. No. __]](SEQ ID NO:238)

The paragraph beginning at page 269, line 13 has been amended as follows:

O-559 [[Seq. I.D. No. __]](SEQ ID NO:239)

The paragraph beginning at page 269, line 16 has been amended as follows:

O-560 [[Seq. I.D. No. __]](SEQ ID NO:240)

The paragraph beginning at page 269, line 19 has been amended as follows:

O-561 [[Seq. I.D. No. __]](SEQ ID NO:241)

The paragraph beginning at page 269, line 22 has been amended as follows:

O-493 [[Seq. I.D. No. __]](SEQ ID NO:242)

The paragraph beginning at page 269, line 25 has been amended as follows:

pLC6G5 [[Seq. I.D. No. __]](SEQ ID NO:243)

The paragraph beginning at page 271, line 18 has been amended as follows:

O-528 [[Seq. I.D. No. __]](SEQ ID NO:244)

The paragraph beginning at page 271, line 21 has been amended as follows:

O-529 [[Seq. I.D. No. __]](SEQ ID NO:245)

The paragraph beginning at page 271, line 24 has been amended as follows:

O-530 [[Seq. I.D. No. __]](SEQ ID NO:246)

The paragraph beginning at page 271, line 27 has been amended as follows:

O-531 [[Seq. I.D. No. __]](SEQ ID NO:247)

The paragraph beginning at page 271, line 30 has been amended as follows:

O-532 [[Seq. I.D. No. __]](SEQ ID NO:248)

The paragraph beginning at page 271, line 33 has been amended as follows:

O-543 [[Seq. I.D. No. __]](SEQ ID NO:249)

The paragraph beginning at page 271, line 36 has been amended as follows:

O-544 [[Seq. I.D. No. __]](SEQ ID NO:250)

The paragraph beginning at page 272, line 1 has been amended as follows:

O-545 [[Seq. I.D. No. __]](SEQ ID NO:251)

The paragraph beginning at page 272, line 4 has been amended as follows:

O-546 [[Seq. I.D. No. __]](SEQ ID NO:252)

The paragraph beginning at page 272, line 7 has been amended as follows:

O-547 [[Seq. I.D. No. __]](SEQ ID NO:253)

The paragraph beginning at page 272, line 10 has been amended as follows:

O-496 [[Seq. I.D. No. __]](SEQ ID NO:254)

The paragraph beginning at page 272, line 13 has been amended as follows:

O-542 [[Seq. I.D. No. __]](SEQ ID NO:255)

The paragraph beginning at page 272, line 16 has been amended as follows:

O-533 [[Seq. I.D. No. __]](SEQ ID NO:256)

The paragraph beginning at page 272, line 19 has been amended as follows:

O-534 [[Seq. I.D. No. __]](SEQ ID NO:257)

The paragraph beginning at page 272, line 22 has been amended as follows:

O-535 [[Seq. I.D. No. __]](SEQ ID NO:258)

The paragraph beginning at page 272, line 25 has been amended as follows:

O-536 [[Seq. I.D. No. __]](SEQ ID NO:259)

The paragraph beginning at page 272, line 28 has been amended as follows:

O-537 [[Seq. I.D. No. __]](SEQ ID NO:260)

The paragraph beginning at page 272, line 31 has been amended as follows:

O-539 [[Seq. I.D. No. __]](SEQ ID NO:262)

The paragraph beginning at page 272, line 34 has been amended as follows:

O-540 [[Seq. I.D. No. __]](SEQ ID NO:263)

The paragraph beginning at page 272, line 37 has been amended as follows:

O-541 [[Seq. I.D. No. __]](SEQ ID NO:264)

The paragraph beginning at page 273, line 1 has been amended as follows:

O-538 [[Seq. I.D. No. __]](SEQ ID NO:261)

The paragraph beginning at page 273, line 4 has been amended as follows:

O-490 [[Seq. I.D. No. __]](SEQ ID NO:265)

The paragraph beginning at page 273, line 7 has been amended as follows:

O-520 [[Seq. I.D. No. __]](SEQ ID NO:266)

The paragraph beginning at page 273, line 10 has been amended as follows:

O-521 [[Seq. I.D. No. __]](SEQ ID NO:267)

The paragraph beginning at page 273, line 13 has been amended as follows:

pHC6G5 [[Seq. I.D. No. __]](SEQ ID NO:268)

The paragraph beginning at page 275, line 26 has been amended as follows:

HC6G5 [[Seq. I.D. No. __]](SEQ ID NO:219)

The paragraph beginning at page 276, line 1 has been amended as follows:

LC6G5 [[Seq. I.D. No. __]](SEQ ID NO:220)

The paragraph beginning at page 288, line 11 has been amended as follows:

Stable IL8RA-expressing transfectants were created using the murine pre-B lymphoma cell line (L1-2) as described (Campbell et al., 1996, *J. Cell Biol.* 134:255-66; Ponath et al., 1996, *J. Exp. Med.* 183:2437-48). All expression constructs were made in pcDNA3 (Invitrogen, CA). Wild-type IL-8RA and IL-8RB cDNA were subcloned into Hind III - Not I and EcoR I - Not I sites, respectively. The second initiation site in the IL-8RB sequence (which corresponds to amino acid sequences of MESDS (SEQ ID NO:417). . .) was used. Forty-eight hours post-transfection, 0.8 my/ml G418 was added and serial dilutions of cells plated in 96-well plates. After 1-2 weeks, G418-resistant cells were stained by appropriate anti-IL-8R antibodies. High level of expression was enriched either by limiting dilution and re-screening or FACS sorting.